RESEARCH PLAN PROPOSAL

ANTIOXIDANT AND CANCER PREVENTIVE PROPERTIES OF *PHALSA (Grewia asiatica)* FRUIT AND ITS LEAVES

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Submitted by

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1. INTRODUCTION

Nutrition may be defined as the science of food and its relationship to health. It is concerned primarily with the part played by nutrients in body growth, development and maintenance. Through centuries, food has been recognized as important for human beings in health and diseases. More recently a great deal of interest has been focused on the role of dietary factors in the pathogenesis of non-communicable diseases such as coronary heart diseases, diabetes and cancer (Park, 2011).

Many compounds have been isolated from plant foods, mainly fruits, green leaves and vegetables, which have been identified to play a protective role in the human system (Dorothy et al., 2013). Natural phenolic phytochemicals in plants have been receiving increased interest from consumers and researchers for their antioxidant activity (Nataraj et al., 2013).

1.1Antioxidants And Their Benefits

Antioxidants are substances that may protect cells from free radical (Shereen, 2014). Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that disarm free radicals, the principal antioxidants are vitamin E, beta-carotene, vitamin C, and selenium. When these antioxidants neutralize free radicals by donating an electron particle, they are left with a small problem. The antioxidants are now missing an electron and have become free radicals themselves. The body cannot manufacture these antioxidants, thus it is important to include a major amount of antioxidants in daily diet (Brown, 2013).

1.1.1 Antioxidants and their role in healthy living

Antioxidant compounds like phenolic acids, polyphenols and flavonoids (anthocyanins), scavenge free radicals such as peroxide, hydroperoxide or lipideroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Scartezzini and Speroni, 2000 and Timberlake and Bridle, 1982). Free radicals are molecules with one or two unpaired electrons. These reactive radicals are important at physiological level to maintain homeostasis in signal transduction and defense against infections (De laminrande et al., 1997). The role of free radicals and active oxygen is becoming increasingly recognized in the pathogenesis of many human diseases including cancer, aging and atherosclerosis (Chen et al., 2005). Humans have evolved a highly sophisticated and complex antioxidant protection system, to neutralize free radicals. These components includes

1.1.1.i Nutrient-derived antioxidants e.g. vitamin C, tocopherols and tocotrienols (vitamin E), carotenoids and aother low molecular weight compounds such as glutathione and lipoic acid.

1.1.1.ii Antioxidant enzymes superoxide, dismutase, glutathione peroxide and glutathione reductase, which catalyze free radicals quenching reactions.

1.1.1.iii Metal binding proteins such as ferritin, lactoferritin, albumin and ceruloplasmin that isolate free iron and copper ions that are capable of catalyzing oxidative reaction.

1.1.2 Role of antioxidants in maintaining food quality

Antioxidants are used in lipid containing foods to minimize rancidity, preventing off flavour, delay the formation of oxidative products, maintain nutritional quality and prolong the shelf life of foods products (Maisuthisakul et al., 2007). The antioxidants play an important role in the manufacturing, packaging and storage of fats and fatty foods and have been proven to retard oxidation (Lin et al., 1981). There is some evidence that antioxidants in plant foods may become especially important as age increases and body produce free radicals. Plants produce hundreds of antioxidants for their own protection. Some that may also be useful to human body are present in vegetables, fruits, herbs and spices, nuts and whole grains. Tea, coffee, extra virgin olive oil, red wine and dark bitter chocolate is also rich in antioxidants. Antioxidants produce the bright colours in fruits and vegetables and the flavor of extra virgin olive oil, tea or coffee. The variety and combination of antioxidants in their natural food sources may also be important as isolated antioxidants may not function in the same beneficial way *(*Stanton, 2012).

1.2 Antioxidants and Their Major Dietary Supplements

Changing lifestyle and environment is led to a great change in the health profile of human population. Higher stress levels among individuals, sedentary lifestyle and deficient diets have increased the onset of degenerative diseases like cancer, diabetes mellitus and cardio vascular diseases. One of the common pathways by which the causative factors initiate or promote the diseases in the human system is through the generation of free radicals. Phenols, polyphenols, flavonoids, terpenes in fruits and vegetables and specific components e.g. lycopene in tomatoes and its products and green tea polyphenols prevent degenerative diseases, offer chemo protection and reduce oxidative stress (Jaganathan et al., 2013). In fruit vitamin A, vitamin C, B-complex group, selenium, phosphorus, etc present in major amount they are rich in antioxidant properties because ample amount of vitamin A, vitamin E, vitamin C, zinc, selenium etc present in fruit and these known as antioxidants in nature. Antioxidants are found in many foods, including fruits and vegetables. Eating a diet with lots of vegetables and fruits is healthy and lowers risks of certain diseases such as heart disease, blood pressure, Alzheimer's disease, cancer, etc. experiments showed that antioxidants interacted with free radicals and stabilized them, thus preventing the free radicals from causing cell damage. The three major antioxidant vitamins are beta-carotene, vitamin C, and vitamin E.

1.2.1 Beta-Carotene

Beta-carotene is converted to vitamin A, an essential nutrient. It has antioxidant activity, which helps to protect cells from damage. Beta-carotene is the main safe dietary source of vitamin A, essential for normal growth and development, immune system function and vision (Nutri facts, 2011). The richest sources of beta-carotene are yellow, orange and green leafy fruits and vegetables such as carrots, spinach, lettuce, tomatoes, sweet potatoes, broccoli and winter squash. In general, the more intense the colour of the fruit or vegetable, the more beta-carotene is present (Bayrel, 2008). The recommended dietary allowance for beta-carotene are, for age 1 to 4, the daily dose is 60-90 mg, age 5 to 8 years, 90-120 mg, age 9 to 12 years, 120-150 mg, age 13 to 16 years, 150-180 mg; and age 16 and older, 180 mg. For more sensitive people to sun the dose of beta-carotene can be increased by 30-60 mg per day for children under 16 years old, and up to a total of 300 mg per day for people older than age 16 (Webmd, 2005).

1.2.2 Vitamin E

Vitamin E is an important vitamin required for the proper function of many organs in the body. It is also an antioxidant; it helps to slow down processes that damage cells. Vitamin E is a vitamin that dissolves in fat. It is found in many foods including vegetable oils, cereals, meat, poultry, eggs, fruits, vegetables, and wheat germ oil. It is also available as a supplement. Vitamin E is also used for cataracts, asthma, and respiratory infections, skin disorders, aging skin, sunburns, cystic fibrosis, infertility, impotence, chronic fatigue syndrome (CFS), peptic ulcers, for certain inherited diseases and to prevent allergies (Webmd, 2005). Vitamin E also protect against the skin effects of chemicals used for cancer therapy (chemotherapy). The Food and Nutrition Board at the Institute of Medicine Recommended Intakes for Individuals for vitamin E. for infants 0 to 6 months: 4 mg/day, for 7 to 12 months: 5 mg/day For children upto 1-3 years: 6 mg/day, 4 to 8 years: 7 mg/day, 9 to 13 years: 11 mg/day. For Adolescents and Adults it is recommended 15 mg/day for 14 and older. For pregnant teens and women it is 15 mg/day and for Breastfeeding teens and women, 19 mg/day. The highest safe level of vitamin E supplements for adults is 1,500 IU/day for natural forms of vitamin E, and 1,000 IU/day for the man-made (synthetic) form (Institute of Medicine, Food and Nutrition Board, 2000)

1.2.3 Vitamin C

Vitamin C plays a huge role in maintaining a healthy lifestyle, and preventing disease. Vitamin C influences the body in a variety of ways. The four most noted functions those vitamins C affect are: antioxidant activity, collagen synthesis. Furthermore, vitamin C is vital for the cardiovascular system. When the body's arteries become clogged, there isn't enough blood flow to the heart which results in a heart attack. Vitamin C's antioxidant properties are absorbed into the blood stream, and repress the accumulation of arterial plaque. Vitamin C can be thought of as a type of "Drain-o" for arteries. It cleans the arteries, and enables a healthy flow of blood to the heart. Vitamin C is extremely important in immune system function because it is involved with T-cells and white blood cells. Vitamin C boosts the white blood cell count; by consuming a high

dose of vitamin C had a less likely chance of contracting diseases. The daily recommended dietary allowances (RDAs) are: Infants 0 to 12 months, human milk content (older recommendations specified 30-35 mg); Children 1 to 3 years, 15 mg; Children 4 to 8 years, 25 mg; Children 9 to 13 years, 45 mg; Adolescents 14 to 18 years, 75 mg for boys and 65 mg for girls; Adults age 19 and greater, 90 mg for men and 75 mg for women; Pregnancy and Lactation: age 18 or younger, 115 mg; ages 19 to 50 years 120 mg. People who use tobacco should take an additional 35 mg per day (Webmd, 2005).

1.3 Fruits: Rich Source of Antioxidants

Table 1

Antioxidant Rich Fruits

Fruits	Beta-carotene	Vitamin E	Vitamin C
Blueberries	47.36µg	4.20mg	14.36mg
Grapefruit	706.56 µg	.85mg	44.03mg
Guava	624 µg	.73mg	228mg
Orange	93.01µg	1.20mg	69.69mg
Avocado	90.52 µg	15.10mg	14.60mg
Strawberries	84.67 µg	2.10mg	84.67mg
Tomatoes	808.20 µg	4.85mg	22.86mg
Bell peppers	1494.08 µg	7.25mg	117.48mg
Sweet potato	13120.26 µg	4.05mg	22.34mg

http://www.whfoods.com/genpage.php?tname=nutrientprofile&dbid=84

1.3.1 Antioxidants in lesser known fruits

Antioxidants in locally available fruits are also present in high amount. These fruits are lesser known and thus underutilized in daily diet. In Rajasthan fruits like *phalsa, ber, karonda, sangria, rasbhari, jungle jalebi, dasra,* etc are lesser known fruits but contain a good amount of antioxidants and help in reducing chronic diseases. A study on Oral pre-treatment with ethanolic extract of the roots of *C. carandas* (ERCC; 100, 200 and 400 mg/kg, po) showed significant hepatoprotective activity against CCl_4 and paracetamol induced hepatotoxicity by decreasing the activities of serum marker enzymes, bilirubin and lipid peroxidation, and significant increase in the levels of uric acid, glutathione, super oxide dismutase, catalase and protein in a dose

dependent manner, which was confirmed by the decrease in the total weight of the liver and histopathological examination. Data also showed that ERCC possessed strong antioxidant activity, which may probably lead to the promising hepatoprotective activities of *C. carandas* root extract. These findings therefore supported the traditional belief on hepatoprotective effect of the roots of *C. carandas* (Hegde et al., 2009).

Chanda and Baravalia, (2010) presented a study on screening of some plant extracts against some skin diseases caused by oxidative stress and microorganisms. *turmeric, mango ginger, asthma plant (dudhi), saptala, hathajori, jungle jalebi, shehtoot, chota khulpa,* were selected to evaluate their antioxidant and antimicrobial potentiality against some skin diseases caused by some bacteria and fungi. The dry powder was extracted in methanol by cold percolation method. Free radical scavenging activities like diphenylpicrylhydrazyl (DPPH), superoxide anion (O2-) scavenging activity, hydroxyl radical (·OH) scavenging activity, reducing capacity assessment and total phenol content were measured. Antimicrobial activity was done against 8 microorganisms by agar well diffusion method. All the plants exhibited a strong correlation between antioxidant activity and total phenol content. *Euphorbia hirta* exhibited best antioxidant activity. The plant extracts showed more activity against Gram positive bacteria and fungi. The best antimicrobial activity was shown by *Euphorbia tirucalli.* The study supports the folkloric use of *E. hirta* and *E. tirucalli* against some skin diseases caused by oxidative stress or by microorganisms.

These locally available fruits, which are lesser, known to people are rich in proteins, carbohydrates, vitamin A, vitamin E and vitamin B etc. To enhance the utility of these fruits and to cure the prevalence of chronic diseases such as heart diseases, blood pressure and cancer, *phalsa* (grewia asiatica) one of the lesser known fruit of Rajasthan has been selected for the present study.

1.3.2 *Phalsa* (*Grewia asiatica*)

The discovery of natural and safe sources of antioxidants of plant origin has increased markedly in recent years, and they have been recognized to be an alternative than synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole and others. However, considering the wide range of plant resources available in the world many of them still remain unexplored. Exploring underutilized resources is of great importance considering their rich nutraceautical value and the presence of bioactive compounds (Bhat et al., 2009). Phalsa is a rich source of nutrients such as proteins, amino acids, vitamins, and minerals and contain various bioactive compounds, like anthocyanins, tannins, phenolics and Flavonoids. Different parts of this plant possess different pharmacological properties. Leaves have antimicrobial, anticancer, antiplatelet and antiemetic activities; fruit possess anticancer, antioxidant, radioprotective and antihyperglycemic properties; while stem bark possesses analgesic and anti-inflammatory activities. (De Feo, 2013)

Phalsa is a plant of known and recognized medicinal value and is used as antibacterial, analgesic, antipyretic, for the treatment of jaundice, skin disorders, diabetes mellitus, anemia, etc (Iqbal et

al., 2005) phalsa is an astringent, coolant and stomatic fruit and a low glycemic index fruit. Phalsa has strong irradiation protection qualities due to its free radical scavenging activity. Such findings were explored in 2009 study by radiological protection and affirmed aging and another in 2008 study by complementary and integrative medicine. Amazingly a 2011 study by "Der Pharma Chmica" found that phalsa fruit and leaves exhibited significant anticancer activities against breast cancer and liver cancer cells. This affirms traditional Native American applications of the fruit against cancer like illness (Marya et al., 2011)

Sharma and Sisodia, (2007) present study on to evaluate the radioprotective effect of *Grewia asiatica* fruit pulp extract (GAE) on Swiss albino mice against radiation induced hematological and biochemical alterations. Swiss albino mice (6–8 weeks) were divided into four groups. Group I (normal) without any treatment. Group II (Drug) was orally supplemented (GAE) once daily at the dose of 700 mg / kg. b.wt / day for 15 days, Group III (control) only irradiated group. GroupIV (Drug+IR) was administered same as group II, then exposed to 5Gy of gamma radiation. Mice were sacrificed at 24 and 72 hours post irradiation. Radiation induced deficit in different blood constituents GSH, GSH-Px, sugar, and protein levels in serum could be significantly increased, whereas radiation induced elevation of lipid peroxidation and cholesterol level was markedly decreased in GAE pre treated animals than control group. Result defines that GAE provides protection against radiation-induced alterations in blood of Swiss albino mice.

Antitumor and in-vitro cytoxicity activity of the methanolic extract of *Grewia asiatica (MEGA).* Study presented by (Kakoti, et al., 2011) showed anticancer activity against Ehrlich's ascites carcinoma (EAC) cell lines. Intraperitoneal administration of 250 and 500mg/kg of MEGA increased the life span of EAC ascitic tumor bearing mice by 41.22 percent and 61.06 percent, respectively. Results were also found in the viable and non viable cell counts after the MEGA treatment. MEGA was assessed for *cytotoxicity activity* against four cancer cell lines and showed 50 percent cytotoxicity to HL – 60, K-562, MCF-7 and Hela cells. MEGA was found to be active in preventing the EAC induced ascites tumor development in mice in a dose dependent manner.
Grewia asiatica, Grewia damine Gaertn and *Grewia* tenax (Forsk) Fiori are medicinally important plants from arid lands of Rajasthan which were screened for the natural products. For the purpose of screening methanolic, ethanolic and ethyl acetate extracts of plants were found better suited for maximum metabolites viz. alkaloids, tannins, glycosides, flavonoids, steroids, terpenoids, phenols, While chloroform fractions of plant parts were contain tannins, saponins, steroids, terpenoids, phenols and resins. Extracts in sterile distilled water were revealed the presence of tannins, anthraquinones, flavonoids and phenols. Stem parts of *Grewia* species found to be richer in metabolites than leaves and callus. The medicinal values of *Grewia* species may be attributed due to the presence of detected metabolites (Sharma and Patni, 2013).

1.3.3 Medicinal uses of *phalsa* (*Grewia asiatica*) fruit

- The *phalsa* fruit relieves inflammations and is used to treat respiratory, heart and blood afflictions and to treat feverish conditions
- The ripe fruit is cooling. It is a wonderful reliever of respiratory ailments like asthma, bronchitis, colds, coughs and sore throat.
- The fruits are a minefield of antioxidants and are useful in reducing cancer risk. They also relieve stomachache.
- The paste of the leaves cures skin infections like eczema and eruptions and heals wounds.
- It brings on urine in cases of stoppage of urine, removes thirst, protects against heat stroke, vomiting, nausea, and uneasy feelings due to heat.
- The fruit relieves liver and gall bladder problems, regulates blood pressure and cholesterol.
- The seeds are used to treat gonorrhoea and fertility problems (Jolly, 2012)
- *Phalsa* has strong irradiation protection qualities due to its amazing free radical scavenging activities. Such findings were explored in a 2009 study and affirmed again in another 2008 study by Complementary and Integrative Medicine(Reddy, 2013)
- According to an article, *phalsa* is a low glycemic index fruit, thereby having positive effects on blood glucose metabolism (Reddy, 2013)

1.3.4 Processing of fruits

Food consists of agricultural products, which are usually seasonal and spoil quickly. To make food available for long time, there are some methods to prolong the storage life of products to preserve them. Fruits provide an abundant and inexpensive source of energy, body-building nutrients, vitamins, minerals and antioxidants. Their nutritional value is highest when they are fresh, but it is not always possible to consume them immediately. Moreover, most fruits are only edible for a very short time, unless they are promptly and properly preserved (James and Kuipers, 2003)

1.3.4 i Blanching- Blanching is done by immersing fruits in water at a temperature of 90-95◦C. By which fruits become soft and enzymes get inactivated (James and Kuipers, 2003). Blanching soften the tissues of fruit, removes air from intercellular spaces, increases density of fruit.

1.3.4.ii Drying- Drying is one of the oldest preservation methods. The moisture level of agricultural products is decreased to 10-15% by drying by which the microorganisms present cannot thrive and the enzymes become inactive. Before drying, the vegetables and fruits have to be thoroughly washed and cut into pieces if necessary. For drying, the relative humidity (RH) of the air should be less than 65%.

1.4 Cancer and Role of Antioxidants in Its Prevention

At the beginning of this century, cancer was the sixth cause of death in industrialized countries and today, it is the second leading cause of death (park, 2011) in India the national cancer registry programme of the ICMR provides data on incidence from populations-based registries and one rural-based population registry at barshi, Maharashtra. It is estimated that there are approximately 2-2.5 million cases of cancer in India at any given point of time with around 7-9 lakh new cases being detected each year.

The superoxide radical responsible for lipid peroxidation and membrane damage are generated from different sources. More over environmental factors like ultraviolet light, ionic radiations, toxic chemicals and metals also cause free radicals formation. Peroxidation of lipid is responsible for damage to tissue and it may be a cause of cancer (Khalad et al, 2011) oxidative damage to DNA, proteins and macromolecules has been implicated in the pathogenesis of a wide variety of diseases most noteably heart diseases and cancer. A growing body of animal and epidemiological studies as well as clinical intervention trials suggests that antioxidants may play a vital role in preventing or slowing the progression of both heart diseases and cancer. Oxidants are capable of stimulating cell division, which is critical factor in mutagenesis. When a cell with damaged DNA strand divides, cell metabolism and duplication becomes deranged, thus a mutation can arise which in turn is an important factor in carcinogenesis. It is believed that antioxidant exert their protective effect by decreasing abnormal increases in cell division. (Percival, 1998)

A number of dietary factors have been implicated in the etiology of cancer, based on experimental research in animals and on epidemiologic studies. Epidemiologic evidence suggests a possible indirect role for dietary factors in certain types of cancer. Laboratories experimentally have been shown that increased susceptibility to chemically induced cancer occurs in vitamin A deficiency and that increased intake of vitamin A exerts a protective effect in most cases (lyon et al., 1983). In laboratory studies vitamin C has been shown to inhibit formation of carcinogenic nitroso compounds, to prevent malignant transformed cells. However, data are inconclusive regarding an important role for vitamin C in prevention of cancer. Non-nutritive compounds such as indoles, flavonoids, phenols and aromatic isothiocynantes in cruciferous vegetable, inhibit chemically induced cancers in laboratory animals (Robinson et al., 1982). Eating fruits and vegetables helps to prevent cancer and this is likely due to the presence of proanthocyanidins. Studies on grape seed extract have also suggested that it reduces breast tumors in rats and skin tumors in mice and it's active in a number of laboratory human cancer cell lines such as skin, breast, colon, lung and stomach and prostate cancer cells (clinical cancer research, 2009)

1.5 Skin Cancer and Role of Antioxidants in Its Treatment

Melanoma and malignant neoplasm derived from melanocytes of the skin and other sites, has been increasing in incidence in worldwide. In the U.S the annual incidence of melanoma has more than doubled from the six cases per 100,000 populations. Data now evolving suggest that the rate of increase of melanoma incidence may be beginning to flatten thought worry persists that continue thinning of the ozone layer or extension of the hole in the ozone layer may lead to further increase in the incidence of the skin cancer melanoma. Melanoma rarely occurs on double-covered areas those on which bressieres and underpants are worn. On the other side, Non-melanoma skin cancer type of melanoma cancer was estimated 100,000 new cases of non-melanoma skin cancer every year in the UK. Non-melanoma cancer spreads faster in the body if not treated timely. Non-melanoma skin cancers usually develop in the outermost layer of skin (epidermis) and the first sign of non-melanoma skin cancer is usually the lump or patch on the skin that does not heal after a weeks of treatment. In most cases, cancerous lumps are red and firm, while cancerous patches are often flat and scaly. The two types of non-melanoma skin cancer are:

- ***Basal cell carcinoma*** – starts in the cells lining the bottom of the epidermis and accounts for about 75% of skin cancers
- ***Squamous cell carcinoma*** – starts in the cells lining the top of the epidermis and accounts for about 20% of skin cancers

Eating certain foods may help protect skin cancer. There are several studies have shown that antioxidants (including vitamin C, vitamin E, beta-carotene and zinc), folic acid, fats and proteins, offer protection from skin cancer. Studies on animals suggest that substances found in foods such as soy and flaxseed may help fight cancer in general (Steven, 2012)

Sies, (2013) discovered the research on the powerful anti-oxidation activity of lycopene, the famous red pigment in tomatoes and other fruits and vegetables. Recent researches with human volunteers showed that lycopene and other carotenoids (natural pigments) effectively ameliorated UV-induced skin damage (erythma) in humans, which consequently helped reduce the risk of skin cancer. Sies discovery increases the possibilities of using dietary intervention for skin cancer protection, and helps the development of functional foods that may enable humans to create a second layer of powerful sunscreen from inside out.

1.6 Significance of the Present Study

There are many lesser known fruits in Rajasthan like *karonda, jungle jalebi, dasra, phalsa, rasbhari* etc. These fruits are rich in antioxidants, having antimicrobial and antibacterial properties. On the basis of the one of the lesser known fruit *phalsa* (Grewia asiatica) has been selected for the present study. *Phalsa* possess a number of therapeutic properties and being rich in antioxidants, and cure chronic diseases such as diabetes, cancer and heart diseases. It also prevents risk of stroke, decreases LDL cholesterol level and blood pressure, cures anaemia, skin

lesion and takes care of poor digestion. In the present study *phalsa* has been selected for estimating antioxidant activity of the fruit itself as well as of its leaves. Further, an effort will made to determine its cancer preventive potential in mice.

1.7 Objectives

- To analyse the proximate composition and antioxidant content (nutrient/ non-nutrient) of *phalsa* (*Grewia asiatica*) fruit and its leaves.
- To determine the antioxidant activity of the fruit and its leaves.
- To assess the cancer preventive potential of the selected dose level of powdered form of *phalsa* (*Grewia asiatica*) fruit and its leaves.

2. METHODOLOGY

The most fundamental step in research is to develop a systematic plan for investigation. Hence this chapter contains relevant information pertaining to the design. It includes the methodological aspect such as selection of tools and other techniques of statistical analysis in order to achieve all objectives of the present investigations. This study will be conducted in 2 phases

2.1 Phase I

Phase I will consist of procurement of *phalsa*(*Grewia asiatica*) fruit and its leaves, their processing (blanching), estimation of macronutrients and antioxidants (nutrient/ non-nutrient) and analysis of antioxidant activity.

2.1.1 Procurement of *phalsa*(*Grewia asiatica*) fruit and its leaves

The fruit and its leaves will be procured from the local market of Jaipur, Rajasthan.

2.1.2 Taxonomy of *phalsa (Grewia asiatica)* fruit and its leaves

Taxonomy of phalsa fruit and its leaves will be done to assure the species of fruit and its leaves.

2.1.3 Processing of *phalsa* (*Grewia asiatica*) fruit and leaves

After procuring the fruit and leaves from the market, both will be washed 2-3 times with running tap water and then will be dried using clean cotton cloth. Thereafter processing will be done in two steps-

2.1.3. i *Blanching*- For blanching the fruit and leaves will be plunged into boiling water, for a minute or two, and finally plunged into iced water or placed under cold running water. Blanching

is will be done to inactivate enzymes; modify texture; preserve color, flavor, and nutritional value; and to remove trapped air (Ralph et al., 2004)

2.1.3. ii *Drying*- The blanched fruits and leaves will be then shadow dried. The fruit will be shadow dried along with its seeds. Shadow drying will be done to avoid nutrient loss from microwave and sun drying. In shadow drying retention of nutrients will be more in comparison to heat drying.

2.1.4 Nutrient estimations of fruit and leaves of *phalsa* (Grewia asiatica)

The fruit and its leaves will be estimated for nutrient (moisture, protein, fat, fiber, carbohydrate and ash) and antioxidants (anthocynanis, beta-carotene, vitamin c and vitamin e) by using standard techniques.

Table 2

Methods to be used for Estimation of Macronutrients

Nutrients	**Methods**	**References**
Moisture	Oven drying method	NIN, 2003
Ash	Muffle furnance method	NIN, 2003
Protein	Micro-kjeldal method	NIN, 2003
Fiber	Acid-alkali treatment	NIN, 2003
Fat	Ether extractive	NIN, 2003
Carbohydrate	Calculation based	NIN, 2003

Table 3

Methods to be used for Estimation of Antioxidants

Antioxidants	**Methods**	**Reference**
Anthocyanins	Chromatography spectrophotometery	Rangana , 2003
Beta-carotene	Spectrophotometery method HPLC	AOAC, (2005)
Vitamin C	Tritremetric method	NIN (2003)
Vitamin E	Colourimetry method HPLC	AOAC, (2005)
Polyphenols	Spectrophotometery method	SWAIN & HILLS, (1959)

2.1.5 Estimation of total antioxidant activity

The total antioxidant activity of fruit and its leaves will be done by using standard technique**.**

Table 4

Method to be used for Estimation of Total Antioxidant Activity

Total antioxidant activity	**Method**	**Refrence**
Total antioxidant activity	DPPH method	Rangana, 2003

2.2 Phase II

Phase II will consist of animal selection, to select an optimum dose level, to develop skin carcinogenesis and experimental design.

2.2.1 Animal selection

The animal experiment will be carried out after the approval of ethical socitety certicficate. After which 6-8 weeks old healthy Swiss albino male mice will be selected.

2.2.2 Dose tolerance study and optimum dose selection

6-8 weeks male adult albino mice will be divided in 6 groups for dose tolerance study. The optimum dose will be selected on the basis of reduction in lipid peroxidation and increase in Glutathione level.

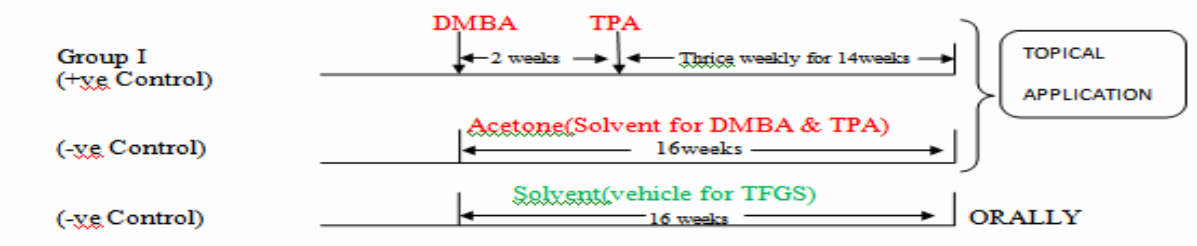
2.2.3 Development of skin cancer (non-melanoma)

DMBA-TPA induced (DMBA once and after 15 days TPA will be given three times in a week for 14 weeks topically to the mice.

2.2.4 Chemo preventive potential of *phalsa*- fruits and its leaves

In this step grouping of mice will be done. In which each group will consist of different aspect.

2.2.4.i Experimental design



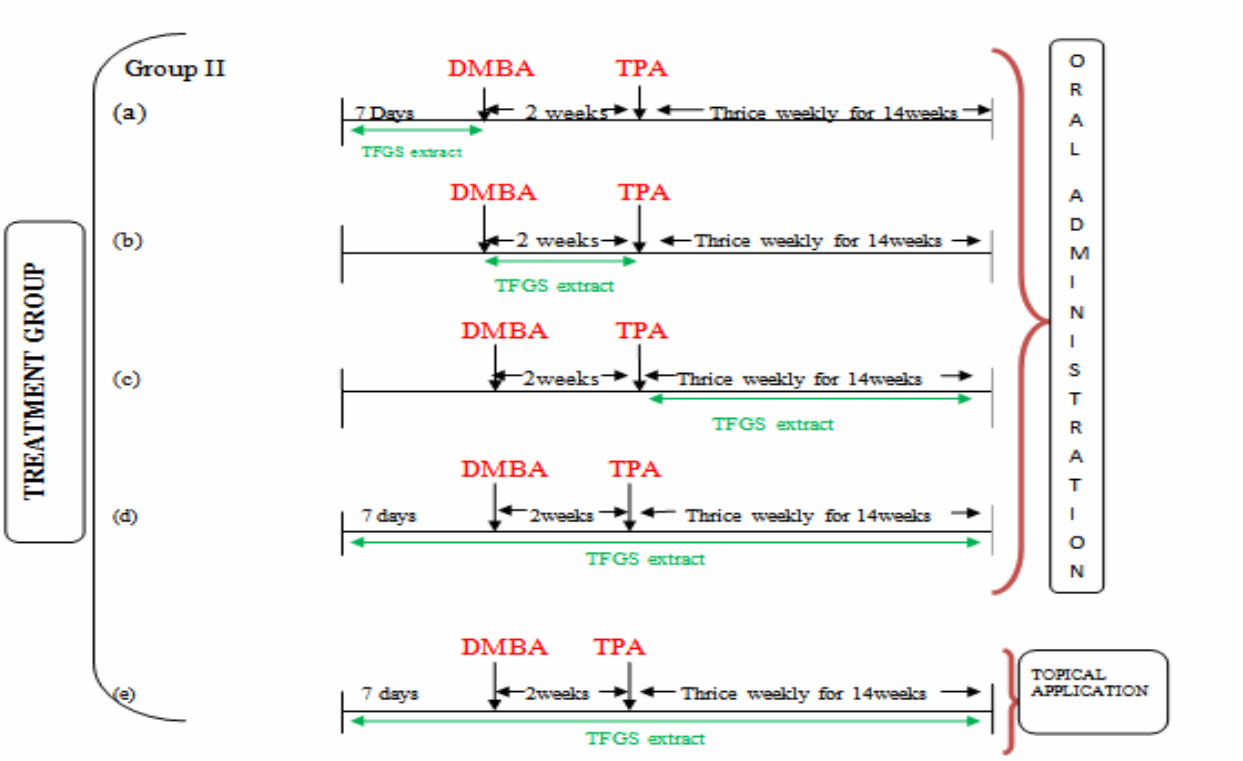


Fig: Dose application pattern in control and treatment group

DMBA: 7,12 Dimethyl benz (a) anthracene

TPA: Tissue plasminogen activator

TFGS: extract of fruit and leaves

GROUP 1: Control group - mice will be kept on only double distilled water (ad libtium)

GROUP 2: *Phalsa* fruit extract treated group

GROUP 3: *Phalsa* leaves extract treated group

GROUP 4: DMBA (7,12dimethyl benz (a) anthracene)-TPA (tissue plasminogen activator) treated group

GROUP 5: Combination group (pre-treated)

GROUP 6: Combination group (post-treated)

GROUP 7: Combination group (throught)

4.2.5 After completion of the experiment biochemical levels of liver antioxidants will be studied (GSH, LPO, CAT)

After the completion of the experimental design the levels of biochemical parameters GSH, LPO and CAT will be studied. Biochemical parameters will be studied in blood sample of mice. By

these biochemical parameters the presence, absence, signs and symptoms of tumor will be identified.

4.2.6 Histopathology of skin tissue

Pathology study of the skin tissue will be done to study the growth of tumor.

4.2.6 Statistical analysis

Statically mean and standard deviation techniques will be used

4.2.7 Thesis writing

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